Instructions: *Use Times New Roman font size 11, 1.0 pt text space, and maximum three A4 pages, including any figures.*

Name or type of instrument	**Equipment package for the development of unique super-resolution and cryo-electron tomography methods**
Unit	Cryo-EM and IMT (KTH)
Platform	CMI
Applicant (Head of Unit)	Marta Carroni
E-mail address	marta.carroni@scilifelab.se
Instrument, acquisition cost (kSEK)	3500
Funding applied for (kSEK)	3500

Description of the instrument

Describe the instrument, its purpose, and its specific capabilities. If possible, provide a link to a web page describing the type of instrument. Does the instrument represent a new technology at the unit, or is it a replacement/upgrade of current equipment base? Is the instrument unique in terms of service to users nationally?

We wish to acquire a system to allow the development of imaging between **super-resolution fluorescence microscopy (SRM)** and **cryogenic electron microscopy (cryo-EM) and tomography (cryo-ET)**. We plan to perform **sequential imaging between live fluorescent super-resolution microscopy and high-resolution cryo-ET (see figure)**. This application would be unique not only in Sweden, but also in the world and we think that at the CMI platform in Stockholm we have the unique combination of instrumentation and skills to drive such development. The acquisition of the expensive instrument requested here, will function as bridging element to the execution of the first experiments necessary to carry out our idea for a **novel imaging method**.

We would like to acquire a PRIMO system (Alveolab, https://www.alveolelab.com/our-products/primo-micropatterning/) combined with a cryo stage fluorescent system from either Leica (Thunder, https://www.leica-microsystems.com/products/thunder-imaging-systems/p/thunder-imager-em-cryo-clem/) or Linkam (https://www.linkam.co.uk/cms196 and https://www.youtube.com/watch?v=ZWVVAf2h2oE). PRIMO would allow us to precisely locate cells to be imaged in specific positions of a cryo-EM grid, making it possible to find them with the fluorescent microscope and to have them in positions suitable for cryo-ET. The associated Leica Thunder system will allow us to perform the first low-resolution fluorescent imaging for preliminary checks before moving the cryo-stage to the MONALISA super-resolution live imaging system (developed in house) and to the commercially available PALM and SIM microscopes available at the IMT (KTH) site, located in the same floor as the cryo-EM unit where the vitrification and cryo-ET will be performed.

The instrument package requested here is a solid piece of equipment and at the same time offers the modularity required for our novel application. In case the system PRIMO+Leica Thunder was deemed too "rigid" for our super-resolution- cryoET imaging modality development, we could instead buy together with PRIMO a Linkam cryostage. While we have a clear quote (attached) for the PRIMO-Leica system, we are still waiting for the same but with the Linkam stage. We estimate (personal communication) that the cost will be similar and **we plan to try obtaining some co-funding from the university (if this one is granted) and to establish a user fee (around 2500sek) per experiment.**

This instrument, combined with the unique plethora of super-resolution microscopes available at the CMI center in Stockholm and with the expertise of the personnel at the cryo-EM and ALM units, will constitute a novel and unique new imaging modality. This represents the possibility for a unique cutting-edge development.

Below, the explanation of the idea in the context of the state-of-the-art correlative techniques.

Cryo-EM has experienced major improvements as new instruments, detectors and software development led to the possibility of achieving near-atomic resolution of **purified molecular assemblies**. While pushing the limit of structural biology in the atomic structure determination of large protein complexes in isolation, it remains important to understand how molecular machines work in the cellular environment. Cryogenic electron tomography (**cryo-ET**) has benefitted from all cryo-EM developments and nowadays much thicker **cellular samples** can be imaged, with better contrast and a near-atomic level of detail. Moreover, cryo-ET can be subsequently combined with **subtomogram averaging**, a procedure similar to single particle analysis, to obtain secondary structure information about molecular machineries imaged *in situ*[1]. We at the cryo-EM unit in Stockholm are expert in

tomogram acquisition and reconstruction (we have projects running in Sweden[2] and Denmark (to be published) and have some good knowledge on subtomogram averaging[3].

Even though cryo-ET offers now high ultrastructural details, identification of the nature of specific structures can be hard when these structures are overall very similar in shape (e.g., cellular skeleton filaments) and previous knowledge is not available. In contrast, **fluorescence microscopy and more specifically super-resolution microscopy**, offers high molecular specificity and can thus provide the identification power lacking in electron microscopy.

In the last decade, the "hybrid" approach of light and electron microscopy (**CLEM - correlative light electron microscopy)** has been used to combine the ability of light fluorescence imaging to obtain large cellular views and having information about the nature of the protein imaged with the high-resolution details of electron microscopy. CLEM and cryo-CLEM, also combined with focused ion beam milling and scanning electron microscopy (FIB-SEM), or other correlative techniques such as CAT (correlative array tomography) are already available at our platform and are applied to a growing number of projects on tissue and cellular analysis.

However, most CLEM technique so far rely on fluorescence imaging at low resolution and on fixed specimens (Figure). When it comes to the analysis of **molecular mechanisms within the cell**, we would instead benefit from **i) the near-atomic localization of small the molecular machines (<20nm), ii) the possibilities of tracking them live and iii) the ability of correlating them with high-resolution electron microscopy to eventually obtain atomic models** (Figure).

i) Super-resolution fluorescence microscopy (SRM) breaks the resolution limits of light and allows the visualization of details as small as 10nm. It exists in many different flavors, most available at our ITM (KTH) unit in Stockholm.

ii) SMR is constantly evolving with the capability of tracking live movement in the cell or in reconstituted systems at high spatial and temporal resolution. Some of these developments are carried at SciLifeLab in the laboratory of fellow Ilaria Testa.

iii) While generic CLEM techniques with low fluorescence resolution and in fixed specimens, have been carried out for few years now and in different laboratories, very few example of correlation between SRM and cryo-ET are available in the literature[4,5] and none about live imaging.

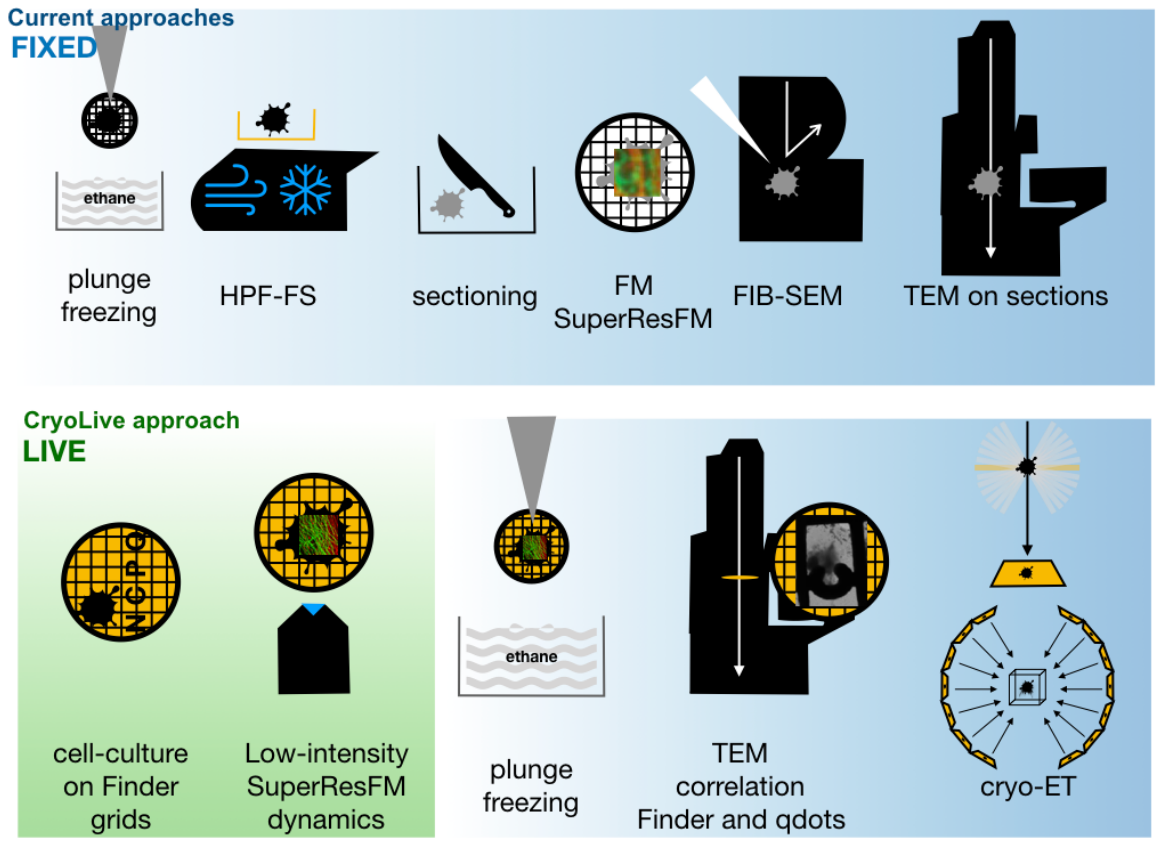

Figure 1 comparison between fixed (up panel) and live (bottom panel) SRM combined with cryo-ET. WE plan to develop a SRM+cryo-ET live method and explore as well other super-resoluton techniques in complex with cryo-ET. We have all the very state-of-the art instrumentation in terms of imaging instrumentation, but we lack the instruments to go from one microscope to the next (cryo-stage and basic preliminary cryo-imaging)

We aim at developing pipelines of imaging between SRM and cryo-ET (cryo-EM), with special interest in live imaging. Main challenges will be: the SRM on cryo-EM grid supports both for live imaging at room temperatures or at cryo/liquid-nitrogen temperatures, the behavior of fluorophores for SRM at cryo temperatures, the freezing of the specimens at desired time points after live imaging and the correlation of the imaging in SRM and cryo-ET.

The acquisition of the PRIMO system combined with cryo-stages and basic fluorescent microscopes will allow us to perform the first tests and to develop a movable stage platform to be used on the SR MONALISA microscope[6] built at SciLifeLab and to the super-resolution single molecule microscopes available at the ITM (KTH) unit. We wish to be able to combine the unique knowledge, instrumentation and accessibility to projects that characterize the Stockholm branch of our CMI platform. The acquisition of the PRIMO-Leica system described above will make it possible. While we can perform the initial proof-of-concept experiments with the personnel currently employed at the cryo-EM and ALM units, in the future, we would like to combine it with a dedicated TDP.

The development of super-resolution microscopy combined with cryo-ET (and cryo-EM) and more specifically of SR live imaging followed by high resolution cryo-ET, will be unique in Sweden and one of the few in the world. So, even though only a handful proof-of-concept projects will benefit from it in the beginning, it will be pivotal for the development and visibility of the SciLifeLab imaging community.

Statement of need

Describe how the instrument will be crucial for the operations, the productivity, the development, and the competitiveness of your unit if obtained. Estimate the need and impact of the instrument from a user perspective. Will the instrument be shared with or used by other units or research groups at SciLifeLab? Also, describe any co-funding plans.

Most attempts at correlation between light and electron microscopy have been carried out using traditional fluorescence microscopy. However, the fluorescence microscopy field has undergone a quantum leap with the advent of super-resolution microscopy that overcomes the diffraction limit of light. Super-resolution fluorescence microscopy, achieved by playing with the photophysical properties of fluorophores, allows resolving details down to 10-20nm. The CMI platform in Solna hosts the ALM Unit with a number of instruments and experts in super-resolution microscopy and the cryo-EM Stockholm Unit, with deep knowledge on atomic cryo-EM and cryo-ET applications. Moreover, the gamma3 floor where the CMI platform is located at SciLifeLab Campus Solna, also hosts the laboratory of fellow Ilaria Testa, international expert in new developments of super-resolution fluorescence techniques, with particular focus on *live imaging*[7].

A natural follow-up for our Unit and Platform would be to work towards the correlation between super-resolution microscopy and cryo-EM. Some types of super-resolution microscopy such as PALM, STED and STORM have been used in correlation with EM. In most of these cases the sample was first "fixed" for EM prior to imaging by fluorescence microscopy, either by chemical fixation, high-pressure freezing followed by resin embedding (Figure1). There are examples of cryo-PALM[8] and we would like to explore more that line as a way to enable the analysis of more subcellular processes. In collaboration with the Testa lab and with the IMT (KTH), we would like to work towards enabling the **super-resolution cryo-EM correlation Live capability** and the "PRIMO + Leica/Linkam stage" will allow us to start working toward this direction. In 2-3 years down the line, this instrument will make possible to try a number of development projects at the interface between cell super-resolution localization and atomic EM details. This would strengthen the competitiveness of our unit and of the whole CMI platform. Support letter from Ilaria Testa and Hans Bloom (head of the IMT-KTH), both experts in the development and application of different types of SRM methodologies are attached.

The acquisition of this instrument by the Stockholm node is not redundant in the general working plan of the CMI platform because of the unique closeness of the ALM and the cryo-EM units and the exposure to a very large number of projects, some of which would immediately benefit from the implementation of cryo-CLEM and superres-cryo-CLEM capabilities.
Moreover, as stated above, **this type of correlative methods is starting to emerge and having the unique live capability will put Sweden at or among those the for-front of the field.**

1. Saibil, H. R. Cryo-EM in molecular and cellular biology. *Mol. Cell* **82**, 274–284 (2022).
2. Villamil Giraldo, A. M. & Kasson, P. M. Bilayer-Coated Nanoparticles Reveal How Influenza Viral Entry Depends on Membrane Deformability but Not Curvature. *J. Phys. Chem. Lett.* **11**, 7190–7196 (2020).
3. Mühleip, A. *et al.* ATP synthase hexamer assemblies shape cristae of Toxoplasma mitochondria. *Nat. Commun.* **12**, 120 (2021).
4. Moser, F. *et al.* Cryo-SOFI enabling low-dose super-resolution correlative light and electron cryo-microscopy. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 4804–4809 (2019).
5. Tuijtel, M. W., Koster, A. J., Jakobs, S., Faas, F. G. A. & Sharp, T. H. Correlative cryo super-resolution light and electron microscopy on mammalian cells using fluorescent proteins. *Sci. Rep.* **9**, 1369 (2019).
6. Masullo, L. A. *et al.* Enhanced photon collection enables four dimensional fluorescence nanoscopy of living systems. *Nat. Commun.* **9**, 3281 (2018).
7. Bodén, A. *et al.* Volumetric live cell imaging with three-dimensional parallelized RESOLFT microscopy. *Nat. Biotechnol.* **39**, 609–618 (2021).
8. Chang, Y.-W. *et al.* Correlated cryogenic photoactivated localization microscopy and cryo-electron tomography. *Nat. Methods* **11**, 737–739 (2014).

Stockholm 2022-09-15

Letter of support

I hereby as head of the Advanced Light Microscopy facility at SciLifeLab and as senior scientist in the Cellular Biophysics group at KTH/Campus Solna, where I as expert in super-resolution florescence microscopy nationally service and develop this imaging modality, support the application from Dr. Marta Carroni to acquire suitable instrumentation for super-resolution and CryoET correlative imaging.

This new advanced imaging modality development and combination is at the global research front of molecular correlative imaging and will give unique national support to all walks of life science. The method development of correlative super-resolution and CryoET imaging is further going to be a fruitful cross-platform collaboration at Campus Solna, which complement cross-site imaging method development in Gothenburg and Umeå within the Cellular and Molecular Imaging platform.

Moreover, the platform management, scientific directors, unit and facility heads of the Cellular and Molecular Imaging platform have all agreed upon this method development and instrumentation application, to develop and extend national support with correlative super-resolution imaging and CryoET at Campus Solna.

Best regards // Hans Blom

Hans Blom (Docent) Royal Institute of Technology - Cellular Biophysics group
Facility Head – Advanced Light Microscopy - Science for Life Laboratory
Phone: +46-(0)70-6882997, Email: hblom@kth.se

SciLifeLab

Stockholm, September 13th, 2022

Dear Marta,

I am happy to offer you my support for the “SciLifeLab Infrastructure Expensive Instrument” application aimed to acquire a cryo-stage fluorescence microscope and PRIMO for cellular imaging with cryo-CLEM.

- This technology will make it possible to start working on the very exciting project we have been thinking about for quite some time: correlation of super-resolution imaging with cryo-ET. Having an instrument to correlate, at cryo temperatures, cellular events that have been previously followed in 3D and across time with our novel RESOLFT (Nat. Biotechnology 2021) and event triggered STED (Nature Methods 2022) methods will actually make live-super-res and cryo-ET correlation possible.

My lab has many on-going projects that would benefit from a further correlative approach with cryo-EM. This would expand the cryo-EM community of your Facility towards more cellular and biophysical projects as well as foster new data driven image analysis development to accurately correlate structural images at different scales.

In summary, I am very excited about this collaboration with you and am looking forward having the possibility to access the cryo-CLEM and PRIMO instrument here at SciLifeLab CS.

All the best, Ilaria Testa

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QUOTATION

The following quotation number should appear in delivery orders, invoices and any other correspondence concerning the order

QUOTATION # QT-AL-2022-108-B

FROM:
Alexane Caignard
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TO:
Dr. Marta Carroni
SciLifeLab CryoEM Facility
Dept. Biochemistry & Biophysics
Stockholm University
Tomtebodavägen 23 A, gamma2
17165, Solna, Sweden

PHOTOPATTERNING PLATFORM - PRIMO 2	
QUOTATION DATE	12/09/2022
VALID UNTIL	11/11/2022

QTY	REFERENCE	DESCRIPTION	UNIT PRICE	TOTAL
1	PRIMO PLATFORM	Photopatterning platform with : - DMD Modulus - Illumination source LED @365nm - Driving electronics - Software LEONARDO - Installation and Training on site (2 days) - Consumables for photopatterning - Customized computer for optimal PRIMO and imaging performances - 1 year of warranty included - High end THUNDER microscope for optimal performances with PRIMO performances and cell culture assay imaging	293 543 €	293 543 €
			TOTAL PRIMO PLATFORM	**293 543 €**
	OPTION:			
1	OKOLAB CAGE INCUBATOR	Incubator for optimal performance with the photopatterning platform PRIMO	34 920 €	34 920 €
1	PRIMOSERV	PREMIUM Service 1 year: 1 year of preventive maintenance after the warranty period including: - Curative maintenance remotly or on site if mandatory - 1 visit for preventive maintenance traveling costs included - Additional training on new features of last software version - Software Update (latest compatible version) during the year - Spare parts	4 550 €	4 550 €
			TOTAL OPTION	**39 470 €**

*Price exc. VAT. Alveole is exempt of VAT, article 262 ter I General Tax Code
Incoterm conditions: EXW
Payment Terms: 30 days upon invoice reception

SUB-TOTAL	333 013 €
FREIGHT AND HANDLING	1 500 €
STANDARD TAX	*
OTHER	
TOTAL	**334 513 €**

Signature